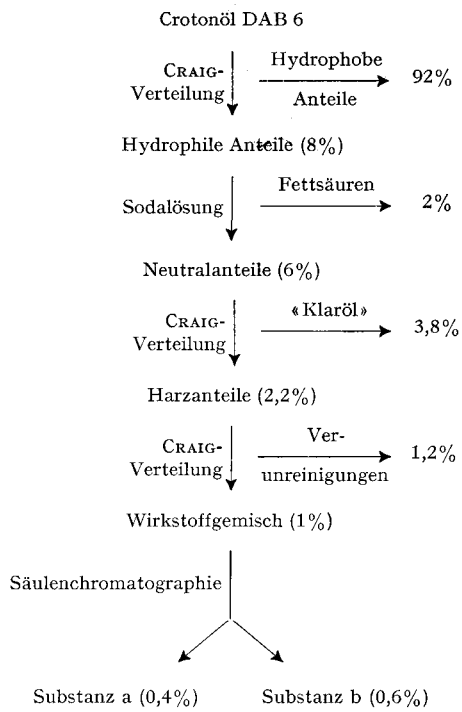

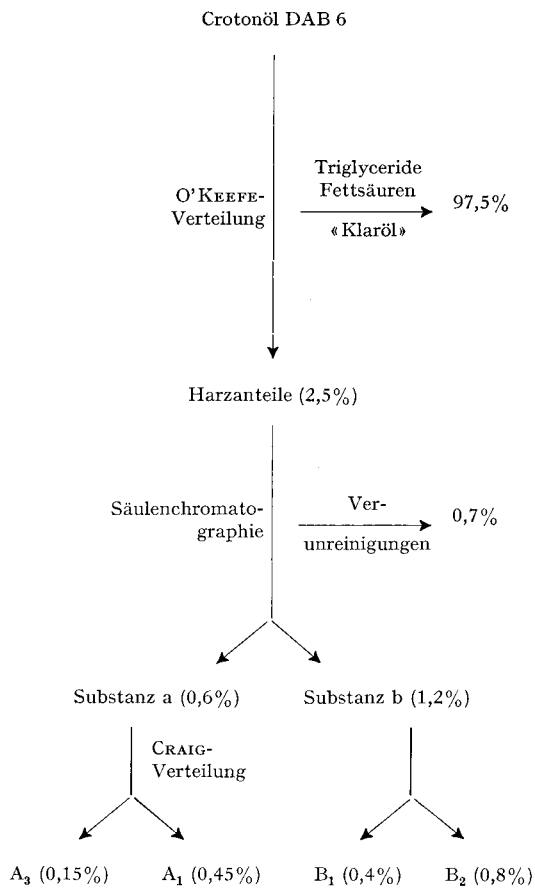

Hecker[6] publizierten, A_1 mit der von Bresch[5] als Ester des Phorbols, $C_{20}H_{28}O_6$[7], erkannten Substanz. Der bisher unbekannte Wirkstoff A_3[8] konnte als Palmitinsäure- und Essigsäureester des Phorbols identifiziert werden. Er besitzt die Bruttozusammensetzung $C_{38}H_{60}O_8$, ist entzündlich, toxisch und cocarcinogen ebenso wirksam wie A_1, B_1 und B_2 und lässt sich wie diese mit *p*-Nitroazobenzolcarbonsäure(4)-chlorid zu kristallisierenden Azoderivaten umsetzen.

Summary. 4 cocarcinogenic compounds have been isolated from croton oil by combination of countercurrent distribution and column chromatography without chemical procedures. The hitherto unknown component A_3 is described as an acetic acid and palmitic acid 'Phorboldiester' with the formula $C_{38}H_{60}O_8$.

J. G. Meyer[9]

Physiologisch-chemisches Institut der Universität, 87 Würzburg (Deutschland), 8. März 1966.

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[8] Erstmals mitgeteilt in Berichten an die Deutsche Forschungsgemeinschaft vom 12.5.64 (B_1 und B_2) und 2.12.64 (A_3).
[9] Der Deutschen Forschungsgemeinschaft danke ich für Personal- und Sachbeihilfen, Fräulein G. Bingmann für ihre Mitarbeit.

Rapid Electron Microscopic Sampling of Single Bacteriophage Plaques

A rapid routine method was developed which permits the visualization by negative staining of bacteriophage particles from single plaques. A tubular piece of glass or metal with at least one well-flattened end, an inner diameter of about 5 mm and a length of about 10 mm is pushed into the agar over the plaque to be investigated. In the well so created, two drops of an aqueous solution of 1% ammonium acetate, 0.8% sucrose and a trace of bovine serum albumin are deposited with a fine pipette. Alternatively 2–4% ammonium acetate solution with a trace of bovine serum albumin, to assist spreading, has been used. The fluid is aspirated and expelled a few times with the same but previously emptied pipette almost in

contact with the plaque surface, especially when the fluid is moving downwards. Aspiration of agar is to be avoided. The resulting suspension is thoroughly mixed with an equal volume of 2% sodium phosphotungstate solution of pH 7.0 in a small polyethylene container. A drop of the mixture is then placed on a collodion-and-carbon coated copper grid and withdrawn immediately with the same pipette or with filter paper. As soon as the preparation has dried, it is examined in an electron microscope with double condenser illumination.

Besides some bacteria, several bacteriophage particles are nearly always found on scanning the first preparation made from the mixture, which is kept at hand until the morphological type of the bacteriophage is documented. Even small plaques usually yield a sufficient number of particles. In this way it is, for instance, possible to differentiate between a mixture of morphologically different bacteriophages causing different plaques on one hand and a morphologically uniform population of bacteriophages causing different plaque types in one and the same Petri dish on the other hand.

Zusammenfassung. Beschreibung einer Präparationstechnik, durch die von einzelnen mit einem kurzen Glas- oder Metallzylinder von der Umgebung abgegrenzten Plaques mit zwei Tropfen Suspensionsmedium genügend Bakteriophagen für eine Untersuchung im Negativkontrastverfahren gewonnen werden.

L. O. Zwillenberg and W. Knapp

Department of Hygiene and Medical Microbiology, University of Berne (Switzerland), February 18, 1966.

Rodent Preimplantation Stages: Mounting 'in toto' and Alterations Due to Anisotonic Solutions

While demonstrating enzyme reactions of the whole egg with the employment of the mounting 'in toto' technique[1] after successful reaction, we observed in more than 2000 examined eggs of the white rat and the golden hamster (*Mesocricetus auratus* Waterhouse), that even slightly anisotonic solutions cause severe alterations in shape and size of the eggs. Concomitant with these alterations are intracellular artefacts, which make it difficult to draw correct conclusions (see Figures 1–3). We had not considered sufficiently this fact in a preliminary note[2].

By performing the reaction on free eggs, as is required for certain enzyme reactions[3], those alterations are avoidable only if exclusively isotonic solutions are used prior to mounting. Deviations of isotonicity frequently occur during mounting with pasting the eggs on a slide, when the small amount of solution surrounding the egg becomes hypertonic through evaporation. Finally, the eggs shrink in spite of good preservation after incubation. Therefore attention must be directed also to a suitable mounting technique.

Mounting procedure. Applying the following procedure, we have succeeded in preparing whole eggs which, after the enzyme reaction with isotonic solutions, differ neither in size nor in shape from recently gained tubal eggs (see Figure 4).

The eggs ready for mounting are transferred in Tyrode's solution or in fixing agent. Preferably, one egg at a time is aspirated by a finely drawn glass pipette and brought on a clean slide coated immediately before mounting with

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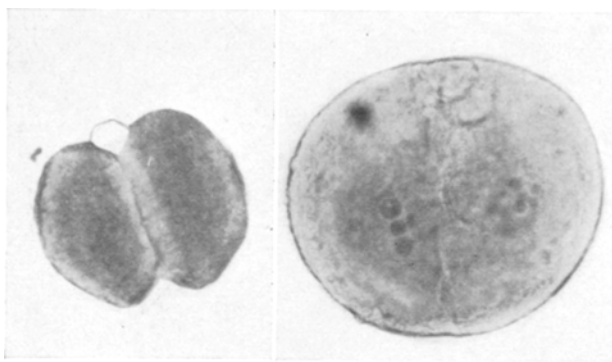
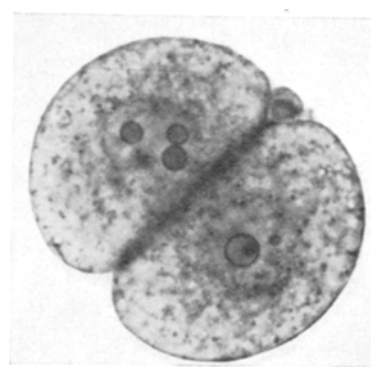


a b

Fig. 1a. 2-cell stage, R 505_3, calculated age 57 h, glucose-6-phosphate dehydrogenase, × 500. Hypertonic incubation solution. Shape rather well preserved. Size excessively diminished. Nucleoli hardly recognizable. Due to artefacts the cleavage plane and the peripheral zones of both blastomeres seem to be free of the reaction product.

Fig. 1b. 2-cell stage, R 7_3, calculated age 57 h, DPNH-diaphorase, × 500. Hypotonic incubation solution. Marked deformation of both blastomeres. Size hardly altered because of subsequent shrinkage in the relatively hypertonic Tyrode's solution. The peripheral zones of both blastomeres seem to contain no reaction product.



c

Fig. 1c. 2-cell stage, R 50_5, calculated age 57 h, DPNH-diaphorase, × 500. Solutions isotonic. Shape and size not altered. Correct distribution of the reaction product.